What Is Claimed Is

1. A method for determining the efficiency of the amplification of a target nucleic acid comprising the steps of:

    a) a dilution series of the target nucleic acid is prepared;

    b) the target nucleic acid is amplified under defined reaction conditions and the amplification is measured in real-time;

    c) a defined signal threshold value is set;

    d) for each dilution the cycle number is determined at which the signal threshold value is exceeded; and

    e) the amplification efficiency is determined as a function of the original amount of target nucleic acid.

2. The method of claim 1, wherein the efficiency of the amplification is determined by:

    a) preparing a dilution series of the target nucleic acid;

    b) amplifying the target nucleic acid under defined reaction and the amplification of the nucleic acid being measured in real-time;

    c) setting a defined threshold value;

    d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

    e) determining a non-linear continuously differentiable function of a logarithm of the copy number of target nucleic acid used for the amplification as a function of the cycle number at which the signal threshold value is exceeded; and

    f) calculating the amplification efficiency E from the function determined in step e).

3. The method of claim 1, wherein the efficiency of the amplification is determined by:

    a) preparing a dilution series of the target nucleic acid;

    b) amplifying the target nucleic acid under defined reaction, the amplification of the nucleic acid being measured in real-time;

    c) setting a defined threshold value;

    d) determining the cycle number for each dilution at which the signal threshold value is exceeded;

    e) determining a non-linear continuously differentiable function of the cycle number determined in step d) as a function of; and

    f) calculating the amplification efficiency E from the function determined in step e).

4.   The method of ⬤ 2, wherein the amplification efficie ⬤ E of a certain original amount of target nucleic acid is determined as the negative local 1st derivative of the continuously differentiable function from step e).

5.   The method of claim 3, wherein the amplification efficiency E of a certain original amount of target nucleic acid is determined as the reciprocal local 1st derivative of the continuously differentiable function from step e).

6.   The method of claim 2, wherein the non-linear continuously differentiable function from step e) is determined with the aid of a polynomial fit preferably of the 3rd, 4th, 5th, 6th or 7th degree.

7.   A method for the absolute quantification of a target nucleic acid in a sample comprising the steps of:
   a) determination of the amplification efficiencies of the target nucleic acid and of an internal or external standard under defined amplification conditions as claimed in claim 1;
   b) amplification of the target nucleic acid contained in the sample and of the internal or external standard under the same defined reaction conditions;
   c) measurement of the amplification of the target nucleic acid and standard in real time; and
   d) calculation of the original copy number in the sample by correction of the copy number derived from step c) with the aid of the amplification efficiencies determined in step a).

8.   A method for the quantification of a target nucleic acid in a sample relative to a reference nucleic acid comprising the steps of:
   a) determination of the amplification efficiencies of the target nucleic acid and of the reference nucleic acid under defined amplification conditions as claimed in claim 1;
   b) amplification of the target nucleic acid contained in the sample as well as of the reference nucleic acid contained in the sample under the same defined amplification conditions;
   c) measurement of the amplification of the target nucleic acid and of the reference nucleic acid in real-time; and
   d) calculation of the original ratio of target nucleic acid and reference nucleic acid in

the sample ● rrection of the ratio derived from st ● with the aid of the amplification efficiencies determined in step a).

9. A method for the relative quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) preparation of a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) amplification of the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, the amplification of the nucleic acid being measured in real-time;

c) setting defined signal threshold values for the target nucleic acid and reference nucleic acid;

d) determining the cycle number Cp to which the signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;

e) determining a continuously differentiable function of the Cp values determined in d) as a function of the logarithm of the amounts used of target nucleic acid and determining a continuously differentiable function of the determined Cp values as a function of the logarithm of the amounts used of reference nucleic acid;

f) determination of the Cp values of the target nucleic acid and reference nucleic acid in the sample to be analysed as well as in a calibrator sample;

g) assignment of the Cp values measured in step f) to particular function values of the functions determined in step e);

h) calculating the quotients of the function values from g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and

i) determination of the ratio of the two quotients from h) as a measure for the original amount of target nucleic acid contained in the sample.

10. A method for the relative quantification of a target nucleic acid relative to a reference nucleic acid and standardized with a calibrator sample comprising the steps of:

a) preparing a common or two separate dilution series of target nucleic acid and reference nucleic acid;

b) amplification of the various dilutions of target nucleic acid and reference nucleic acid under defined reaction conditions, the amplification of the nucleic acid being measured in real-time;

c) setting defi●signal threshold values for the target ●eic acid and reference nucleic acid;

d) determining the cycle numbers Cp at which signal threshold values defined for the target nucleic acid and reference nucleic acid are exceeded in each dilution;

e) determining a continuously differentiable function of the logarithm of the amounts used of target nucleic acid as a function of the Cp values determined in d) and determining a continuously differentiable function of the logarithm of the amounts used of reference nucleic acid as a function of the determined Cp values;

f) determining the Cp values of the target nucleic acid and reference nucleic acid in the sample to be analysed as well as in a calibrator sample;

g) assignment of the Cp values measured in step f) to particular function values of the functions determined in step e);

h) calculating the quotients of the function values from g) of the target nucleic acid and reference nucleic acid for the sample to be analysed as well as for the calibrator sample; and

i) determination of the ratio of the two quotients from h) as a measure for the original amount of target nucleic acid contained in the sample.

11. The method of claim 10, wherein the continuously differentiable functions from step e) are determined with the aid of a polynomial fit preferably of the 3rd, 4th, 5th, 6th or 7th degree.

12. The method of claim 10, wherein the amplified nucleic acids are detected with the aid of at least one fluorescent-labelled hybridization probe.

13. The method of claim 12, wherein the amplified nucleic acids are detected with the aid of FRET hybridization probes, molecular beacons or TaqMan probes.

14. The method of claim 10, wherein the amplified nucleic acids are detected with the aid of a DNA-binding dye, preferably with SybrGreen I.